Review

A survey of antiviral drugs for bioweapons

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Smallpox (variola major), and the haemorrhagic fever viruses (filoviruses and arenaviruses) are classified as Category A biowarfare agents by the Centers for Disease Control. Category A agents pose a significant risk to public health and national security because they can be easily disseminated by aerosol, although with the exception of variola, they are not easily transmitted from person to person. An attack with these viruses would result in high morbidity and mortality and cause widespread panic. With the exception of smallpox and Argentine haemorrhagic fever virus, there are no vaccines or approved treatments to protect against these diseases. In this review we focus on promising prophylactic, therapeutic and disease modulating drugs (see Figure 1 for select chemical structures).

***Keywords*: orthopoxvirus, filovirus, arenavirus, biodefence, bioterrorism, category A**

Introduction

Bioweapons are a threat to global health. Unlike nuclear and chemical weapons, virus-based biological weapons (BW) are part of the natural global flora and fauna, with one notable exception, smallpox. In this review we will focus on inhibitors of arenaviruses, poxviruses, and filoviruses. In general there are three types of inhibitors needed for effective biodefence: i) Prophylactic treatments which enable the individual to resist infection and would help limit the spread of a BW agent. Drugs of this type are scarce and of the class A viruses, only members of the *Orthopoxvirus* genus have promising prophylactic drug candidates. ii) Therapeutic treatments that inhibit viral replication, reduce viral load and consequently limit the degree of mortality and morbidity. iii) Disease modifiers that do not act directly against the virus but rather target aspects of the viral pathogenesis and act to transform the host into an inhospitable environment for viral replication. These different classes of compounds can either be specific to the virus or broad-acting. Broad-acting compounds are attractive because there would be fewer compounds to stockpile as contingencies. In all cases, compounds need to be orally available to facilitate the rapid distribution and dosing of huge numbers of people in case of a large-scale attack.

Infection with BW agents can be significantly different than an infection with their counterparts in nature. These agents may be delivered in ways that the natural virus is not usually transmitted and possibly at infectious doses that are many times what occurs in nature. These nonstandard routes may produce diseases that are distinct from natural forms of the disease. To further complicate the problem, these viruses could be genetically engineered to overcome vaccines, therapeutics and detection. The combination of these alternatives with a few viruses significantly amplifies the issues surrounding drug discovery for BW agents.

Understanding what types of compounds are effective at inhibiting viral replication will aid in developing drugs that address possible genetic modifications. An effective antiviral serves another important role aside from limiting replication during an infection. In its stockpiled form, it may discourage use of a particular BW agent. Furthermore, because some of these viruses are endemic in different parts of the world, the ability to effectively treat the infections they cause will be a significant boon to global public health.

Orthopoxviruses

A wealth of work has been done in a variety of systems to find drugs with antiviral activity against members of the orthopoxvirus genus, and many drugs have been identified that target different parts of the viral life cycle. Orthopoxviruses are the largest known animal viruses and encode some 200 different gene products (Moss, 2001). They replicate in the cytoplasm of infected cells and are not dependent upon the host cell nucleus. Possible targets for these viruses are the DNA polymerase, virus-encoded immune modulators, structural genes and host factors

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14. ABSTRACT

Smallpox (Variola major), and the haemorrhagic fever viruses (filoviruses and arenaviruses) are classified as Category A biowarfare agents by the Centers for Disease Control. Category A agents pose a significant risk to public health and national security because they can be easily disseminated by aerosol, although with the exception of variola, they are not easily transmitted from person to person. An attack with these viruses would result in high morbidity and mortality and cause widespread panic. With the exception of smallpox and Argentine haemorrhagic fever virus, there are no vaccines or approved treatments to protect against these diseases. In this review we focus on promising prophylactic, therapeutic and disease modulating drugs (see Figure 1 for select chemical structures).

15. SUBJECT TERMS

orthopox virus, filovirus, arenavirus, biodefense, bioterrorism, Category A agents

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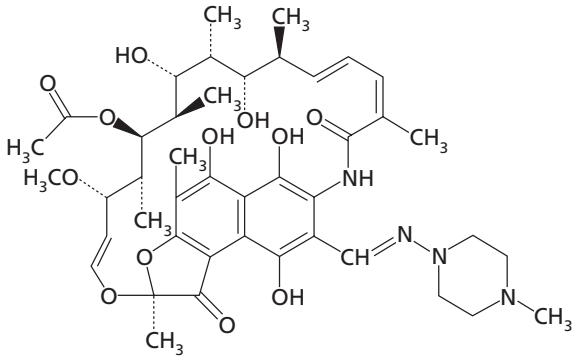
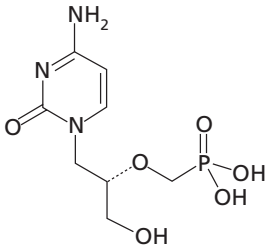
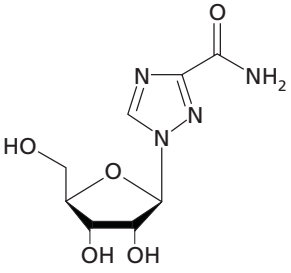
Figure 1. Chemical structures of selected antiviral drugs



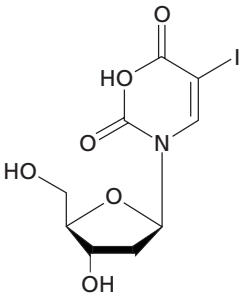
Rifampicin[1]



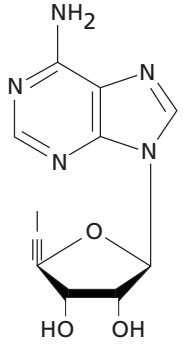
Cidofovir[1]



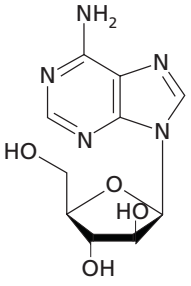
Ribavirin[1,3]



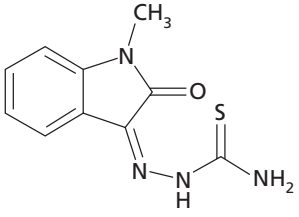
5-Iodo-dUrd[1]



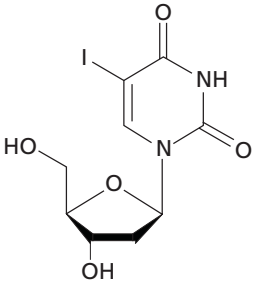
6′-Iodo acetylenic Ado[1,2]



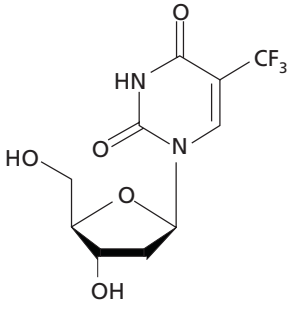
Adenine Arabinoside[1]



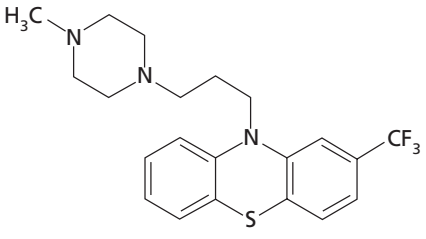
Methisazone[1,3]



Idoxuridine[1]



Trifluridine[1]



Trifluoperazine[3]

Structures of small molecules that exhibit activity against orthopox viruses[1], filoviruses[2] or arenaviruses[3]. Some of these compounds have broad antiviral activity as noted by the superscripts following the name of the compound.

necessary for replication. Most of the drugs that display anti-orthopox activity are therapeutic agents. There are currently no known orthopox-disease modifiers.

Prophylactic treatments

Rifampicin blocks viral assembly by interacting with the product of the D13 gene of poxviruses (Sodeik *et al*., 1994). Rifampicin inhibited tail lesions in mice with 1 dose of 250 mg/kg 6–24 h before infection, or repeated doses for 5 days starting 5 days before infection. Interferon and polyacrylic (interferon inducer) acid were shown to be effective in the tail lesion model if given 24 h before vaccinia virus (VV) infection (De Clercq & De Somer, 1968). Interferon was also effective in keratitis in monkeys if treatment was started 15 h before viral challenge (Neumann-Haefelin *et al*., 1975).

Cidofovir

The group of drugs known as acyclic nucleoside phosphonates contains the most promising anti-pox drugs investigated to date. (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine [(S)-HPMPA] is phosphorylated by cellular enzymes to an active di-phosphate form which then acts as an inhibitor of viral DNA synthesis (Votruba *et al*., 1987). It has a broad range of activity against DNA viruses including adeno-, herpes-, hepadna-, irido-, and poxviruses (De Clercq *et al*., 1986). (S)-HPMPA is effective against VV replication at an inhibitory concentration 50% (IC_{50}) of 0.3 μg/ml (De Clercq *et al*., 1986) and it suppressed vaccinia tail lesion formation in mice at a dose of 5–100 mg/kg/day. (S)-HPMPC (cidofovir) has an activity spectrum similar to (S)-HPMPA, however it is less toxic, so it has surpassed its adenine counterpart in development as an antiviral drug (De Clercq, 1987). Using a neutral red uptake assay, Baker *et al*. (2003) found that cidofovir is effective against over 30 different strains of variola virus with IC_{50}s below 30 μg/ml.

Cidofovir has been licensed (as Vistide) for treating cytomegalovirus (CMV) retinitis in human immunodeficiency virus (HIV)-infected patients but is being investigated for treating other DNA virus infections, namely infections of poxviruses (De Clercq, 1996; De Clercq, 2003). (S)-HPMPC also suppressed tail lesion formation in normal and SCID VV-infected mice. It suppressed viral replication in the liver, lungs, kidneys and brain while preventing death (Neyts & De Clercq, 1993). Death was delayed if given 7 days before infection with VV (Neyts & De Clercq, 1993). Cidofovir (100 mg/kg) given subcutaneously on days 0, 2 and 4 post-infection resulted in 90–100% survival after an intransal or aerosol infection of mice with cowpox (Bray *et al*., 2000). Given 16 days before challenge or 4 days after, a single dose of 100 mg/kg protected 50% of infected mice (Bray *et al*., 2000, Smee *et al*., 2000). Cidofovir was effective in monkeys exposed by aerosol to monkeypox with a single treatment of 5 mg/kg on the day of infection (Huggins *et al*., 1998).

Molluscum contagiosum, a skin disease caused by a poxvirus that can be severe in HIV-infected patients, resolved completely after intravenous or topical treatment with cidofovir (Meadows *et al*., 1997; Davies *et al*., 1999). Although cidofovir is a promising anti-pox drug, a significant limitation is that it is poorly absorbed when administered orally (Wachsman *et al*., 1996), which would limit its utility in a medical emergency. Studies by Hostetler *et al*. (1997) showed that derivatization of acyclovir with 1-*O*-hexadecyl-glycero-3-phosphate provided a compound that was 100% orally bioavailable in mice. For this reason, Kern *et al*. (2002) developed a series of ether lipid analogues of cidofovir and tested their efficacy and oral bioavailability in a lethal aerosol ectromelia (mouse pox) model in mice (Buller *et al*., 2004). In addition to having *in vitro* IC_{50}s that were between 11- and 60-fold better than cidofovir, the ether lipids significantly reduced mortality rates in mice. Treatment with a 3 mg/kg oral dose of the most potent analogue, octadecyloxyethyl-CDV (ODE-CDV) immediately before viral exposure resulted in a 10% mortality rate as compared to 100% in control mice and mice treated with unmodified cidofovir. Treatment with 5 mg/kg on days 0–4 protected 100% (16 of 16) of animals from death.

Therapeutic treatments

Ribavirin. Ribavirin (RBV) is in a class of drugs known as inosine monophosphate (IMP) dehydrogenase inhibitors. It has a relatively broad spectrum of antiviral activity, inhibiting both DNA and RNA viruses (Sidwell *et al*., 1972). IMP dehydrogenase converts IMP to XMP. This step is crucial in the synthesis of the purine mononucleotides. RBV inhibits IMP dehydrogenase by RBV 5′-monophosphate (Streeter *et al*., 1973) and depletes GTP and dGTP. Baker *et al.* (2003) found RBV to inhibit three strains of variola in Vero cells in a neutral red uptake assay at an IC_{50} of 18.4 μg/ml.

RBV has already been shown to be effective in several animal models such as topical treatment of VV keratitis in rabbits (Sidwell *et al*., 1973). It is also effective against tail lesion formation in intravenous infections with VV at 4, 20 or 100 mg/kg/day for 4 days immediately after infection (De Clercq *et al*., 1976). In a case report of a single patient, RBV used in conjunction with immune globulin stopped the spread of VV in an immunocompromised individual (Kesson *et al*., 1997). This however was not a controlled experiment so a cause-and-effect relationship between RBV treatment and improvement in the patients' condition could not be established. Several related compounds such as FICAR (De Clercq *et al*., 1976), EICAR (De Clercq *et al*., 1991), tiazofurin (Kirsi *et al*.,

1983) and selenazole (Kirsi *et al*., 1983) have similar antiviral activities.

Thymidylate synthase inhibitors. Thymidylate synthase inhibitors inhibit the thymidine synthase step that converts dUMP to dTMP. This depletes dTTP pools and therefore inhibits DNA synthesis. This class of drugs has been studied in the treatment of herpes simplex infections and VV infections, but they are more active against VV (De Clercq, 1980). Several 5-substituted 2′-deoxyuridines like 5-fluoro-dUrd and 5-nitro-dUrd are the most potent and have an IC_{50} of 0.1–0.2 μg/ml. The iodo-, ethyl-, and thiocyano-counterparts prevented tail lesions from VV at 4, 20 or 100 mg/kg for 7 days in mice (De Clercq *et al*., 1976). Unfortunately, these drugs are more apt to inhibit host cell DNA synthesis than viral DNA synthesis (De Clercq, 2001).

Other nucleoside analogues. There is another group of nucleoside analogues that do not interact directly with thymidylate synthase, but instead interfere with the viral DNA polymerase. Adenine arabinoside (Ara-A) is one of these nucleoside analogues and is the most promising of this class of drugs. The 5′-triphosphate of Ara-A competes with dATP for incorporation into DNA strands. Ara-A does inhibit cellular DNA synthesis but unlike the thymidylate synthase inhibitors, it does so at a level that is 20-fold higher than the IC_{50} for VV (De Clercq *et al*., 1980). Ara-A inhibited rabbit keratitis (Sidwell *et al*., 1975) and prevented formation of VV lesions in rabbits by topical or systemic administration (Smee & Sidwell, 2003). Ara-A also prevented VV tail lesion formation in mice given 300 mg/kg/day subcutaneously for 7 days. Ara-A prevented death when given intracranially to mice with rabbitpox at 31–1000 mg/kg/day for 7–9 days beginning 1 day before or 1 day after infection (Dixon *et al*., 1968; Sidwell *et al*., 1968). Cytosine arabinoside (Ara-C) is another nucleoside analogue that inhibits cellular and viral DNA synthesis at the same concentration and is therefore not a good drug candidate (De Clercq *et al*., 1980). In addition Ara-C failed to protect SCID mice in a lethal VV infection (Neyts *et al*., 2002).

Some branch-chain sugar nucleosides like 3′-C-methyladenosine and 3′-C-methylcytidine have anti-VV activity at a dose of 2 mg but the work was never followed up (Walton *et al*., 1969). A series of alkylated adenosine analogues were tested and 8-methyladenosine was shown to be a potent and selective inhibitor of VV at an IC_{50} of 0.2 μg/ml, which is a concentration that is non-toxic to cells in cell culture (Van Aerschot *et al*., 1993). These compounds, however, had no therapeutic effect on disease progression in cases of uncomplicated smallpox (Dennis *et al*., 1974; Koplan *et al*., 1975). One of the analogues, S2242, was active against almost all herpesviruses and it inhibited VV at an IC_{50} of 0.4 μg/ml (Neyts *et al*., 1994). This compound was fully protective against VV infection when given as an ester prodrug in normal and SCID mice (Neyts & De Clercq, 2001). The mode of action is unknown. Neyts *et al*. (2002) also showed that 5′-iodo-2′deoxyuridine, another inhibitor of herpesvirus replication, delayed death in a lethal VV infection of SCID mice, even when treatment was delayed up to 4 days after infection.

SAH hydrolase inhibitors. S-adenosylhomocysteine (SAH) hydrolase inhibitors also exhibit a broad range of activity, inhibiting orthopoxviruses, bunyaviruses, arenaviruses, rhabdoviruses, paramyxoviruses, and reoviruses (De Clercq, 1987). Viruses that are inhibited by these compounds are dependent on methylations where S-adenosyl-methionine (SAM) is the methyl donor. SAH is a product and inhibitor of the SAM-dependent methyltransferase reactions and must be removed by SAH hydrolase for the methylations to proceed correctly. This plays a role in the 5′-cap formation and maturation of VV mRNA (Borchardt, 1980). Those that have marked activity against VV are C-c^3Ado (De Clercq and Montgomery, 1983), neplanocin A and C (De Clercq, 1985), 3-deazoneplanocin A (De Clercq *et al*., 1989; Tseng *et al*., 1989), DHCeA and c^3DHCeA (De Clercq *et al*., 1989; Hasobe *et al*., 1987) and its saturated derivatives, F-C-Ado (Cools *et al*., 1991), 5′-norasteromycin (Patil *et al*., 1992; Siddiqi *et al*., 1994; Siddiqi *et al*., 1995), R-6′-C-methylneplanocin A (Shuto *et al*., 1996), 6′-homoneplanocin A (Shuto *et al*., 1996), 2-fluoroneplanocin A (Obara *et al*., 1996), and 6′-iodo acetylenic Ado (Robins *et al*., 1998). These inhibitors all inhibit VV in cell culture at an IC_{50} of less than 1 μg/ml with little to no cytotoxicity at the active concentrations. The most potent carbocyclic adenosine analogues that inhibit at an IC_{50} of 0.1 μM (~0.03 μg/ml) are 3-deazaneplanocin A, DHCaA, c^3DHCaA, -5-noraristeromycin, and (R)-6′-C-methylneplanocin A. 3-Deazaneplanocin was effective against VV tail lesion formation at doses of 4–8 mg/kg/day given subcutaneously for 7 days starting 1 day before infection (Tseng *et al*., 1989).

OMP decarboxylase inhibitors. Orotidine monophosphate (OMP) decarboxylase inhibitors prevent conversion of OMP to uridine monophosphate (UMP) and lead to a depletion of UTP and CTP pools. CTP synthase inhibitors block conversion of UTP to CTP and therefore also deplete CTP pools. Because this group of compounds inhibits RNA synthesis, they would not affect non-dividing cells and would show specific antiviral activity. The OMP inhibitor, pyrazofurin, inhibits several viruses, including VV at an IC_{50} of 0.1 μg/ml, which is non-toxic to cells (De Clercq, 2001). The CTP inhibitors,

carbodine and cycolpentenyl cytosine, are good candidates for drug development because of a low IC_{50} (0.02 μg/ml) and no reported cell toxicity. In spite of the apparent potency and lack of toxicity of this class of compounds, relatively little work has been done to evaluate their potential in treatment of pox infections.

Other therapeutic treatments. There are a few other types of drugs that show some promise as anti-pox drugs but there are few data regarding their efficacy. Polyanionic substances such as sulfated polysaccharides inhibit virus binding to cells (Baba *et al*., 1988a; Mitsuya *et al*., 1988) and are effective against enveloped viruses like HIV, herpes simplex virus (HSV), CMV, vesicular stomatitis virus (VSV), respiratory syncytial virus (RSV), togaviruses and arenaviruses (Witvrouw & De Clercq, 1997). They also inhibit VV, although not as well with IC_{50}s of 4–20 μg/ml (Baba *et al*., 1988b; Witvrouw & De Clercq, 1997). Thiosemicarbazones have activity against several families of viruses such as adeno-, herpes-, picorna, reo-, arbo- and myxoviruses (Bauer, 1971). Studies indicate that they interfere with viral maturation (Woodson & Joklik, 1965). IC_{50}s of thiosemicarbazones range from 1–60 μg/ml (Bauer, 1971; Safrin *et al*., 1997). In animal models, they are active against tail lesions in mice at 6–400 mg/kg/day for 7 days in mice infected intravenously with VV (Boyle *et al*., 1966). In several studies under various conditions, thiosemicarbazones were also able to prevent death in mice infected intracerebrally with rabbitpox virus (Thompson *et al*., 1953; Sidwell *et al*., 1968; Dixon *et al*., 1968). Field trials where thiosemicarbazones were administered as prophylactic agents against smallpox have been conducted (Heiner *et al*., 1971; Bauer *et al*., 1969; Bauer *et al*., 1963; do Valle *et al*., 1965; Rao *et al*., 1969).

In West Pakistan, Heiner *et al*. (1971) identified individuals that had contact with patients infected with smallpox virus but had not yet developed the disease themselves. These individuals were divided into treatment and placebo groups, and the compound methisazone was administered in doses according to the age of the individual. The subjects were then monitored for incidence of smallpox. In agreement with Rao *et al*. (1969), the authors did not observe a significant difference in incidence of smallpox in treated individuals. In contrast Bauer *et al*. (1963) conducted a similar study in Madras, India and found that out of 2292 treated contact cases, there were 6 cases of smallpox and 2 deaths. Out of the 2710 untreated contacts, there were 113 cases and 21 deaths. These results agreed with a study conducted by do Valle in Sao Paulo, Brazil (do Valle *et al*., 1965).

Although these studies demonstrated the protective capability of methisazone, it should be noted that the majority of individuals that participated in these studies were vaccinated soon after exposure (Heiner *et al*., 1971). Thus these trials represent the effect of post-exposure vaccination and methisazone combined. N_1-isonicotinyl-N_2-3-methyl-4-chlorobenzoylhydrazine (IMCBH) inhibits assembly by targeting a 37 kDa protein which is a component of the golgi-derived viral envelope.

A study was done in 2003 (Kern, 2003) that, in addition to testing efficacy of cidofovir and its derivatives and prodrugs, focused on testing drugs that are already available for treating other virus infections. These drugs were tested for their ability to limit replication of VV or cowpox virus in cell culture. Of the already licensed drugs cidofovir, Gemcitibine, Idoxuridine, Trifluridine, and Vidaribine were all active against both viruses, with IC_{50}s under 50 μM. Cidofovir, as mentioned previously, was licensed for use in CMV infections but has problems with toxicity over long-term use. However, long-term toxicity is not likely to be a problem when treating poxvirus infections with cidofovir, as the drug seems to work well with only a few doses. In addition, Probenicid, a drug that decreases toxicity of many different drugs, can be used to combat the toxic side effects of cidofovir. Gemcitabine is licensed for use in cancer therapy and is also very toxic. Idoxuridine and Trifluridine are used for topical treatment of HSV infections but do not have a good toxicology database. Vidarabine is also used in HSV infections but administered parenterally. Although it displayed significant activity in cell culture, it was not active in murine models for VV or cowpox.

In addition to available drugs, Kern (Kern, 2003) also tested drugs with a Phase II/Phase III history for anti-pox activity in cell culture. Of these, only two compounds, adefovir dipivoxil and fialuridine, had good activity with IC_{50}s between 0.24 and 13 μM. Adefovir dipivoxil is a nucleoside phosphonate like cidofovir. It is indicated for treating HIV and has good oral bioavailability and low toxicity. Fialuridine, however, has proven to be significantly toxic in treatment of hepatitis in humans.

Filoviruses

The filoviruses (Ebola and Marburg viruses) are single-stranded, negative-sense RNA viruses. Some of the drugs that were already shown to have antiviral activity against other families of viruses were tested for their ability to inhibit filovirus replication. However, most of the significant work has come from novel approaches. Possible targets for filoviruses are the RNA-dependent RNA polymerase, the interferon antagonist, entry functions of the glycoprotein, structural protein/protein interactions and key virus host factors that affect replication. It is known that infected individuals with lower peak viral titres have improved outcomes (Towner *et al*., 2004).

Therapeutic treatments

SAH hydrolase inhibitors. Huggins *et al.* (1999) tested a series of SAH hydrolase inhibitors for anti-Ebola activity *in vitro* and in a lethal mouse model. Inhibitors of the enzyme, as previously mentioned, indirectly inhibit transmethylation reactions by a feedback mechanism. Transmethylation is required for replication of viruses that cannot initiate mRNA synthesis by the so-called cap-stealing process. It is thought that, in spite of the fact that SAH inhibitors target a cellular enzyme, they selectively inhibit viral methylation events at a lower concentration than that which would be required to inhibit cellular methylation. SAH inhibitors were tested for their ability to inhibit filovirus replication in a monolayer of Vero E6 cells. CaAdo, Ca-c^{3}Ado, Npc A, c^3-Npc A, DHCpAdo, DHCp-c^{3}Ado and DDFA all had IC_{50}s of less than 65 μM. In addition, these same drugs, except for CaAdo and Npc A, had negligible toxicity at the highest concentrations tested. Ca-c^3 Ado was administered to BALB/c mice that were infected intraperitoneally with 30 LD_{50} of Ebola virus. Treatment begun 1 day before infection with 0.7–20 mg/kg every 8 h for 9 days protected 100% of mice. Additional studies revealed that SAH hydrolase inhibitors cause significantly increased production of IFN-α in infected mice (Bray *et al.*, 2002). This suggests that a second mode of action of SAH hydrolase inhibitors is to boost the immune response by stimulating interferon production.

Because SAH hydrolases may protect mice by inducing interferon, then it follows that direct treatment with interferon should also confer protection. Recombinant IFN-α was able to protect mice against infection with Ebola Zaire when begun up to 2 days after viral challenge and continued for 5–7 days post-infection (Bray & Paragas, 2002). The interferon inducer, polyICLC, was also protective in this model. Exogenous IFN-α, however, did not protect guinea pigs (Sergeev *et al.*, 1997). In these animals, treatment delayed but did not prevent death. In a study of rhesus macaques, treatment with human IFN-α given immediately after infection with Ebola Zaire resulted only in a 2-day delay in the onset of illness and death (Jahrling *et al.*, 1999). There is speculation that the SAH hydrolase inhibitors worked well because they induced production of the innate IFN-α (Bray & Paragas, 2002). It is not clear whether the induction of interferon was a result of the drug or simply a normal response from the host after an abortive infection caused by the drug treatment. Filoviruses appear to be sensitive to the effects of type 1 interferon.

Cyanovirin-N (CV-N) is a protein that is produced by cyanobacteria and it binds to high-mannose oligosaccharides. Barientos *et al.* (2003) found that the presence of 0.12 μM CV-N in the medium of Ebola-infected Vero cells reduced cytopathic effects and allowed uptake of control amounts of neutral red. In Ebola-infected mice, daily treatment with 0.48–5.6 mg/kg of CV-N for 6 days (starting day -1 or day 0) delayed the onset of illness and prolonged the time of survival – however, it failed to protect mice from death. The potency of CV-N in cell culture was corroborated by Barrientos *et al.* (2004).

Potential treatments. Recently, much work has been done on enveloped viruses and late domains. All retroviruses tested to date and several other enveloped viruses require one of three amino acid motifs called late domains (PTAP, PPXY, YXXL) (Demirov & Freed, 2004) to interact with components of the cellular trafficking machinery and achieve efficient egress from infected cells. Ebola has an overlapping PTAPPEY motif (Licata *et al.*, 2003; Harty *et al.*, 2000). As components of the cellular trafficking machinery, these late domain interacting proteins are regulated by the process known as ubiquitination. It is known that monoubiquitination often serves as a signal for the internalization and sorting of membrane-associated proteins into the endosomal pathway. Several lines of evidence suggest a connection between ubiquitination and enveloped virus release (Vogt, 2000). Among them are: i) Several retroviruses contain free, virion-associated ubiquitin (Putterman *et al.*, 1990), and ii) proteasome inhibitors can inhibit the release of some retroviruses (Patnaik *et al.*, 2000; Schubert *et al.*, 2000; Ott *et al.*, 2002; Accola *et al.*, 2000). Inhibition of these late domain interactions and/or the ubiquitination events that might regulate them make attractive targets for antiviral therapy. Although dominant negative forms of these late domain interacting/cellular trafficking proteins inhibit viral release in cell culture, they have yet to be tested in an animal model (Demirov & Freed, 2004).

Disease modifiers. There have been very few reports of attempts to modulate filovirus disease rather than inhibiting the viral life cycle. One promising report came from Geisbert *et al.* (2003a). In a previous report they found that the disease triggered by Ebola virus in primates results in hyper-activation of the coagulation cascade (Geisbert *et al.*, 2003b). Specifically, Ebola causes over-expression of tissue factor in monocytes and macrophages. This over-expression leads to recruitment of other clotting factors to cells expressing tissue factor, leaving them in short supply elsewhere in the host. This lack of clotting factors is what leads to disseminated intravascular coagulation, thrombosis related failure and vascular haemorrhage (Arai *et al.*, 2000). Geisbert *et al.* (2003a) attempted to control this hyper-activation by blocking the clotting pathway. Recombinant nematode anticoagulant protein c2 (rNAPc2) directly inhibits the tissue factor complex by binding to factor X (Vlasuk & Rote, 2002). An initial study

showed that of three rhesus monkeys treated with rNAPc 24 h after exposure to Ebola, one survived and the other two died on days 11 and 14 post-infection while the control animal died on day 8.

The animal that survived lived for more than 1 year after viral challenge. In a second, more in-depth, study, two control animals died within 9 days of exposure while of the six treated animals, four died on days 8, 10, 13 and 14, and two survived and remained healthy for more than 9 months. Although rNAPc2 is not thought to have any effect on the virus itself, viral titres were significantly lower in animals that survived. In addition, rNAPc2 did not display any toxicity within the range tested.

Ignatyev *et al*. (2000) treated Marburg-infected guinea pigs with anti-tumour necrosis factor (TNF)-α serum. Treatment beginning on day 3 post-infection resulted in survival of three out of five animals. In another report, treatment with a TNF-α inhibitor (desferoxamin) resulted in survival of three out of six animals (Ignat'ev *et al*., 1996).

Arenaviruses

Just as in the case of the filoviruses, several drugs that have already been studied for activity against other viruses were tested for inhibition of the haemorrhagic fever-causing arenaviruses. The most efficacious compound was RBV. Arenaviruses may be targeted for discovery of inhibitors of viral entry and/or replication.

Therapeutic treatments

Ribavirin. Jahrling *et al*. (1980) tested the efficacy of RBV *in vitro* and in rhesus monkeys. *In vitro* studies showed that RBV was effective in inhibiting viral replication in Lassa virus-infected cells. Inhibition was dependent upon dose of RBV, the multiplicity of infection and cell type. RBV was more potent in rhesus alveolar macrophages than in Vero cells. Six out of ten untreated monkeys infected subcutaneously with Lassa virus died. In the same study, all eight RBV-treated monkeys survived. A treatment regimen of an initial subcutaneous injection of 50 mg/kg followed by 10 mg/kg injections every 8 h through to day 18 was effective. It made little difference if treatment began on day 0 or 5 days after infection, although monkeys that began treatment on day 5 experienced a moderately severe disease.

McCormick *et al*. (1986) published a study that showed RBV to be effective for treating humans with Lassa fever at varying stages of the disease. Patients admitted to one of two participating hospitals in Sierra Leone were evaluated for risk factors associated with Lassa fever. Patients with an aspartate aminotransferase (AST) level of greater than or equal to 150 units per litre were randomly separated into treatment groups. The presence of Lassa antigen in these patients was confirmed by an immunofluorescence assay with a Lassa virus-specific antibody and viral titres were performed on 10-fold dilutions of the specimens. In untreated Lassa fever patients with serum levels of greater than or equal to $10^{3.6}$TCID$_{50}$ (tissue culture infectious dose), a 76% (46 total cases) fatality rate was observed. In patients with similar viral loads and treated with a 2 g loading dose of RBV and 1 g every 6 h for 4 days, only 32% (31 total cases) and 30% (10 total cases) of patients treated intravenously or orally respectively died. McCormick *et al*. (1986) also found that treatment was most effective if initiated on or before the sixth day of illness.

Weissenbacher *et al*. (1986a; 1986b) tested the effect of different doses of RBV on Junin-infected marmosets. All doses tested inhibited viral replication. Treatment with 15 mg/kg twice daily, a 75 mg/kg loading dose followed by 25 mg/kg daily and a 75 mg/kg loading dose followed by 25 mg/kg daily in conjunction with 2 ml of immune serum all protected ≥50% of infected animals, while all six control animals died. Unexpectedly, RBV combined with immune serum did not protect better than RBV alone. Animals that were not infected but treated with RBV developed severe anaemia and leucopenia which was similar to that present in infected RBV treated animals, but more pronounced than that induced by virus alone.

In a lethal model of intracerebrally Junin-infected 10-day-old rats, a single dose of 60 or 90 mg/kg of RBV injected intracerebrally 2 h before infection led to 40% survival. Intraperitoneal infection of 2-day-old rats is also usually lethal. Animals received five 30 mg/kg doses intraperitoneally at 24 h intervals starting 2 h before inoculation. Up to 73% of treated animals survived versus 22% in control groups (Remesar *et al*., 1988).

McKee *et al*. (1988) tested RBV in rhesus macaques. All (four) untreated animals died between 21 and 26 days after intramuscular infection with Junin virus. Laboratory animals were either treated prophylactically or therapeutically. The prophylactically treated group received 60 mg/kg/day on days 0–3, 30 mg/kg/day on days 4–7, and 15 mg/kg/day on days 8–17. All of these animals survived infection with a mild disease course. Animals were killed 6 months after infection and three out of four had no pathological signs of disease. In contrast, three out of four animals that were given 60 mg/kg on day 6 post-infection and 15 mg/kg/day on days 7–20 died. Mean time of death was slightly delayed in comparison to untreated animals although one died particularly early on day 8. The three remaining animals in this group actually recovered from clinical illness and were healthy until about day 28 post-infection, when they developed tremors and signs of aggression. Signs of central nervous system (CNS) damage progressed in two of the three monkeys and they died on days 36 and 43 post-infection. This neurological pathology

persisted in the remaining monkey until the ninth week post-infection. The last monkey was killed 6 months post-infection and all three of the monkeys that developed nervous system damage displayed pronounced lesions in the CNS. As seen in other studies, all animals that received RBV in this study had side effects that included thrombocytosis and severe anaemia. Side effects disappeared soon after cessation of drug treatment.

The Maiztegui group also published a study on the utility of RBV in Junin virus outbreaks (Enria & Maiztegui, 1994). In a double-blind study, Junin virus-infected patients were either given placebo or 34 mg/kg of RBV as a loading dose followed by 17 mg/kg every 6 h for 4 days and 8 mg/kg every 8 h for the following 6 days. Patients that received placebo had a 33.3% mortality rate while patients receiving RBV had a mortality rate of only 12.5%. The only side effect seen in RBV-treated patients was anaemia, which was easily managed.

In the autumn of 1994, three patients living in northern Bolivia presented to hospital officials with symptoms consistent with Bolivian haemorrhagic fever (BHF) (Machupo virus infection). A Centers for Disease Control and Prevention (CDC) team (Kilgore *et al*., 1997) was called in to evaluate the therapeutic use of RBV in these patients. The first patient died on the fourth day after admission, before diagnosis with BHF, and was unable to receive therapy with RBV. Diagnosis with BHF followed post-mortem. Patients two and three lived and worked together. Patient two was not diagnosed and treated with RBV until 12 days after admission to the hospital. Therapy continued for 19 days and the patient eventually recovered. BHF was confirmed by detection of viral antigen in the serum. Patient three did not become ill until more than 3 weeks after patient two. Because of the similar symptoms and the association with patient two, treatment with RBV was began only 3 days after admission to the hospital, before the presence of Machupo virus was confirmed. Patient three completely recovered from a significantly less severe disease after 10 days of treatment. Although these three cases of BHF were not part of a designed experiment and there were no controls, the use of RBV in humans is still noteworthy.

Stampidine. Stampidine is a nucleoside analogue that has strong antiviral activity against HIV-1 and HIV-2 (Uckun *et al*., 2004), and is not associated with any noticeable toxicity in mice or rats whether given as a single intravenous dose or as an intraperitoneal injection daily for 8 weeks (Uckun *et al*., 2003). Uckun *et al*. (2004) evaluated the ability of stampidine to inhibit Lassa virus. CBA mice were intracranially injected with 1000 plaque-forming units of the Josiah strain of Lassa Virus. Mice were then given placebo or treated with a series of injections (24 h before; 1 h before; and 24, 48, 72 and 96 h after inoculation) of stampidine at 25 or 50 mg/kg. All of the 18 control mice developed sickness and 13 died between days 8 and 11. All eight mice given 25 mg/kg of stampidine also developed sickness but only two died on days 8 and 10. Of the 10 mice treated with 50 mg/kg of stampidine, only one developed any physical signs of sickness and eventually died on day 16. The remaining nine animals remained healthy through the 21 days of observation.

Other therapeutic agents. Candurra *et al*. (1996) did a study based on the fact that many events that take place at the plasma membrane, like endo- and exocytosis and changes in cell morphology, can be influenced by a group of drugs called phenothiazines. These same events are involved at different stages of enveloped virus–cell interactions. Trifluoperazine (TFP) and chlorpromazine (CPZ) both significantly inhibited the IV4454 strain of Junin virus in Vero cells with IC_{50}s of 10.1 μM and 12.5 μM, respectively. Cell toxicity was determined and was low for both compounds. TFP had a 50% cytotoxic concentration (CC_{50}) of 61.9 μM and CPZ had a CC_{50} of 81.4 μM. Both compounds were also tested for antiviral activity against more pathogenic strains of Junin virus and had similar IC_{50}s. TFP and CPZ were also similarly active against Tacaribe virus while Pichinde virus was less susceptible to both compounds. Time of addition or removal experiments showed that CPZ was most active within the first 3 h of infection while TFP maintained maximal activity for up to 7 h post-infection.

Meliacine, a peptide isolated from leaves of *Melia azedarach* L was shown to have anti-Junin activity by Castilla *et al*. (1998). Meliacine had no toxicity within the range tested (0.048 μg/ml to 50 μg/ml) while inhibiting Junin replication in Vero cells with an IC_{50} of 0.13 μg/ml when cells were pre-treated and an IC_{50} of 0.94 μg/ml when the peptide was added after cells were exposed to the virus. Castilla *et al*. also found that Meliacine inhibits Junin virus replication at two different steps, the first when the virus is uncoated and the second while infectious particles are released.

Junin virus, along with several other members of the arenavirus genus, contains an 11 kDa protein called Z or p11 whose function is unknown. What is known, however, is that this protein contains a conserved Cys-His-Cys RING finger motif that binds two zinc ions. Other studies (Rice *et al*., 1996; Tummino *et al*., 1997; Witvrouw *et al*., 1997) have shown that certain compounds target the Zn-finger motifs of HIV-1 and cause the zinc ions to be ejected from the protein. This results in loss of the native structure and inhibition of virus replication. Garcia *et al*. (2000) tested some of these Zn-finger-targeted compounds for inhibition of Junin virus infection in Vero cell monolayers.

Fifteen compounds were tested and only three had significant antiviral activity and low toxicity. NSC20625, 3-7, and 2-71 had 50% effective concentrations (EC_{50}s) of 9.3 μM, 6.5 μM and 8.9 μM, respectively; 3-7 had the best toxicity profile with a CC_{50} of 363.7 μM, 2-71 came in second at 257.7 μM and NSC20625 had a CC_{50} of 94.4 μM. Although the CC_{50} of NSC20625 was the lowest, it was still 10 times higher than the EC_{50}.

The same group (Garcia *et al*., 2003a) tested the antiviral activity of several thiosemicarbazones in the same manner. Twenty-three compounds were tested but only six (1a, 4a, 6a, 8a, 10a, and 11a) showed a selectivity index (CC_{50}/EC_{50}) of 10 or more.

In a 2003 paper, Garcia *et al*. (2003b) identified two azoic compounds that displayed anti-arenavirus activity. These two compounds, ANNB and AB, had EC_{50}s of less than 35 μM for Junin virus and Tacaribe virus and CC_{50}s of greater than 360 μM in Vero cells. The authors suggest that the compounds are likely to affect the process of intracellular virion assembly as they do not exert their effects until late in the viral life cycle.

Conclusions

There are many compounds that have promise for inhibiting viral replication *in vitro*. However, many of these compounds are lacking the necessary pharmokinetics, bioavailability and toxicity testing that can transform promising *in vitro* drug leads into potential candidates for advanced development and testing. The effective translation of *in vitro* results into antiviral therapies is a daunting task. There are far fewer antiviral compounds than parasite treatments or antibiotics. This may be due in part to the complex life cycle of viruses that is interwoven with host cellular function. The unique nature of viral BW agents may necessitate novel approaches to address these ever-present threats.

Disclaimer

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the US Army.

References

Accola MA, Strack B & Gottlinger HG (2000) Efficient particle production by minimal Gag constructs which retain the carboxy-terminal domain of human immunodeficiency virus type 1 capsid-p2 and a late assembly domain. *Journal of Virology* **74:**5395–5402.

Arai A, Hirano H, Ueta Y, Hamada T, Mita T & Shirahata A (2000) Detection of mononuclear cells as the source of the increased tissue factor mRNA in the liver from lipopolysaccharide-treated rats. *Thrombosis Research* **97:**153–162.

Baba M, Pauwels R, Balzarini J, Arnout J, Desmyter J & De Clercq E (1988a) Mechanism of inhibitory effect of dextran sulfate and heparin on replication of human immunodeficiency virus *in vitro*. *Proceedings of the National Academy of Sciences of the United States of America* **85:**6132–6136.

Baba M, Snoeck R, Pauwels R & De Clercq E (1988b) Sulfated polysaccharides are potent and selective inhibitors of various enveloped viruses, including herpes simplex virus, cytomegalovirus, vesicular stomatitis virus, and human immunodeficiency virus. *Antimicrobial Agents and Chemotherapy* **32:**1742–1745.

Baker RO, Bray M & Huggins JW (2003) Potential antiviral therapeutics for smallpox, monkeypox and other orthopoxvirus infections. *Antiviral Research* **57:**13–23.

Barrientos LG, Lasala F, Otero JR, Sanchez A & Delgado R (2004) *In vitro* evaluation of cyanovirin-N antiviral activity, by use of lentiviral vectors pseudotyped with filovirus envelope glycoproteins. *Journal of Infectious Diseases* **189:**1440–1443.

Barrientos LG, O'Keefe BR, Bray M, Sanchez A, Gronenborn AM & Boyd MR (2003) Cyanovirin-N binds to the viral surface glycoprotein, GP1,2 and inhibits infectivity of Ebola virus. *Antiviral Research* **58:**47–56.

Bauer DJ (1971) Chemotherapy of virus infections. *Proceedings of the Royal Society of Medicine* **64:**544–546.

Bauer DJ, St Vincent L, Kempe CH, Young PA & Downie AW (1969) Prophylaxis of smallpox with methisazone. *American Journal of Epidemiology* **90:**130–145.

Bauer DJ, St Vincent L, Kempe CH & Downie AW (1963) Prophylactic treatment of small pox contacts with N-methylisatin beta-thiosemicarbazone (Compound 33t57, Marboran). *Lancet* **35:**494–496.

Borchardt RT (1980) S-Adenosyl-L-methionine-dependent macromolecule methyltransferases: potential targets for the design of chemotherapeutic agents. *Journal of Medicinal Chemistry* **23:**347–357.

Boyle JJ, Haff RF & Stewart RC (1966) Evaluation of antiviral compounds by suppression of tail lesions in vaccinia-infected mice. *Antimicrobial Agents and Chemotherapy* **6:**536–539.

Bray M, Martinez M, Smee DF, Kefauver D, Thompson E & Huggins JW (2000) Cidofovir protects mice against lethal aerosol or intranasal cowpox virus challenge. *Journal of Infectious Diseases* **181:**10–19.

Bray M & Paragas J (2002) Experimental therapy of filovirus infections. *Antiviral Research* **54:**1–17.

Bray M, Raymond JL, Geisbert T & Baker RO (2002) 3-deazaneplanocin A induces massively increased interferon-alpha production in Ebola virus-infected mice. *Antiviral Research* **55:**151–159.

Buller RM, Owens G, Schriewer J, Melman L, Beadle JR & Hostetler KY (2004) Efficacy of oral active ether lipid analogs of cidofovir in a lethal mousepox model. *Virology* **318:**474–481.

Candurra NA, Maskin L & Damonte EB (1996) Inhibition of arenavirus multiplication *in vitro* by phenotiazines. *Antiviral Research* **31:**149–158.

Castilla V, Barquero AA, Merisch SE & Coto CE (1998) *In vitro* anti-Junin virus activity of a peptide isolated from *Melia azedarach* L. leaves. *International Journal of Antimicrobial Agents* **10:**67–75.

Cools M, Balzarini J & De Clercq E (1991) Mechanism of antiviral and cytotoxic action of (+/-)-6' beta-fluoroaristeromycin, a potent inhibitor of S-adenosylhomocysteine hydrolase. *Molecular Pharmacology* **39:**718–724.

Davies EG, Thrasher A, Lacey K & Harper J (1999) Topical cidofovir for severe molluscum contagiosum. *Lancet* **353:**2042.

De Clercq E (1980) Antiviral and antitumor activities of 5-substituted 2'-deoxyuridines. *Methods and Findings in Experimental and Clinical Pharmacology* **2:**253–267.

De Clercq E (1985) Antiviral and antimetabolic activities of neplanocins. *Antimicrobial Agents and Chemotherapy* **28:**84–89.

De Clercq E (1987) S-adenosylhomocysteine hydrolase inhibitors as broad-spectrum antiviral agents. *Biochemical Pharmacology* **36:**2567–2575.

De Clercq E (1996) Therapeutic potential of Cidofovir (HPMPC, Vistide) for the treatment of DNA virus (i.e. herpes-, papova-, pox- and adenovirus) infections. *Verhandelingen - Koninklijke Academie Voor Geneeskunde Van Belgie* **58:**19–47; discussion 47–49.

De Clercq E (2001) Vaccinia virus inhibitors as a paradigm for the chemotherapy of poxvirus infections. *Clinical Microbiology Reviews* **14:**382–397.

De Clercq E (2003) Clinical potential of the acyclic nucleoside phosphonates cidofovir, adefovir, and tenofovir in treatment of DNA virus and retrovirus infections. *Clinical Microbiology Reviews* **16:**569–596.

De Clercq E, Cools M, Balzarini J, Marquez VE, Borcherding DR, Borchardt RT, Drach JC, Kitaoka S & Konno T (1989) Broad-spectrum antiviral activities of neplanocin A, 3-deazaneplanocin A, and their 5'-nor derivatives. *Antimicrobial Agents and Chemotherapy* **33:**1291–1297.

De Clercq E, Cools M, Balzarini J, Snoeck R, Andrei G, Hosoya M, Shigeta S, Ueda T, Minakawa N & Matsuda A (1991) Antiviral activities of 5-ethynyl-1-beta-D-ribofuranosylimidazole-4-carboxamide and related compounds. *Antimicrobial Agents and Chemotherapy* **35:**679–684.

De Clercq E & De Somer P (1968) Effect of interferon, polyacrylin acid, and polymethacrylic acid on tail lesions on mice infected with vaccinia virus. *Applied Microbiology* **16:**1314–1319.

De Clercq E, Descamps J, Verhelst G, Walker RT, Jones AS, Torrence PF & Shugar D (1980) Comparative efficacy of antiherpes drugs against different strains of herpes simplex virus. *The Journal of Infectious Diseases* **141:**563–574.

De Clercq E, Holy A, Rosenberg I, Sakuma T, Balzarini J & Maudgal PC (1986) A novel selective broad-spectrum anti-DNA virus agent. *Nature* **323:**464–467.

De Clercq E, Luczak M, Shugar D, Torrence PF, Waters JA & Witkop B (1976) Effect of cytosine, arabinoside, iododeoxyuridine, ethyldeoxyuridine, thiocyanatodeoxyuridine, and ribavirin on tail lesion formation in mice infected with vaccinia virus. *Proceedings of the Society for Experimental Biology and Medicine* **151:**487–490.

De Clercq E & Montgomery JA (1983) Broad-spectrum antiviral activity of the carbocyclic analog of 3-deazaadenosine. *Antiviral Research* **3:**17–24.

Demirov DG & Freed EO (2004) Retrovirus budding. *Virus Research* **106:**87–102.

Dennis DT, Doberstyn EB, Awoke S, Royer GL Jr & Renis HE (1974) Failure of cytosine arabinoside in treating smallpox. A double-blind study. *Lancet* **2:**377–379.

Dixon GJ, Sidwell RW, Miller FA & Sloan BJ (1968) Antiviral activity of 9-beta-D-arabinofuranosyladenine. V. Activity against intracerebral Vaccinia virus infections in mice. *Antimicrobial Agents and Chemotherapy* **8:**172–179.

Do Valle LA, De Melo PR, De Gomes LF & Proenca LM (1965) Methisazone in prevention of variola minor among contacts. *Lancet* **2:**976–978.

Enria DA & Maiztegui JI (1994) Antiviral treatment of Argentine hemorrhagic fever. *Antiviral Research* **23:**23–31.

Garcia CC, Brousse BN, Carlucci MJ, Moglioni AG, Martins Alho M, Moltrasio GY, D'Accorso NB & Damonte EB (2003a) Inhibitory effect of thiosemicarbazone derivatives on Junin virus replication *in vitro*. *Antiviral Chemistry and Chemotherapy* **14:**99–105.

Garcia CC, Candurra NA & Damonte EB (2000) Antiviral and virucidal activities against arenaviruses of zinc-finger active compounds. *Antiviral Chemistry and Chemotherapy* **11:**231–237.

Garcia CC, Candurra NA & Damonte EB (2003b) Differential inhibitory action of two azoic compounds against arenaviruses. *International Journal of Antimicrobial Agents* **21:**319–324.

Geisbert TW, Hensley LE, Jahrling PB, Larsen T, Geisbert JB, Paragas J, Young HA, Fredeking TM, Rote WE & Vlasuk GP (2003a) Treatment of Ebola virus infection with a recombinant inhibitor of factor VIIa/tissue factor: a study in rhesus monkeys. *Lancet* **362:**1953–1958.

Geisbert TW, Young HA, Jahrling PB, Davis KJ, Kagan E & Hensley LE (2003b) Mechanisms underlying coagulation abnormalities in ebola hemorrhagic fever: overexpression of tissue factor in primate monocytes/macrophages is a key event. *Journal of Infectious Diseases* **188:**1618–1629.

Harty RN, Brown ME, Wang G, Huibregtse J & Hayes FP (2000) A PPxY motif within the VP40 protein of Ebola virus interacts physically and functionally with a ubiquitin ligase: implications for filovirus budding. *Proceedings of the National Academy of Sciences of the United States of America* **97:**13871–13876.

Hasobe M, McKee JG, Borcherding DR & Borchardt RT (1987) 9-(trans-2',trans-3'-Dihydroxycyclopent-4'-enyl)-adenine and -3-deazaadenine: analogs of neplanocin A which retain potent antiviral activity but exhibit reduced cytotoxicity. *Antimicrobial Agents and Chemotherapy* **31:**1849–1851.

Heiner GG, Fatima N, Russell PK, Haase AT, Ahmad N, Mohammed N, Thomas DB, Mack TM, Khan MM, Knatterud GL, Anthony RL & McCrumb FR Jr (1971) Field trials of methisazone as a prophylactic agent against smallpox. *American Journal of Epidemiology* **94:**435–449.

Hostetler KY, Beadle JR, Kini GD, Gardner MF, Wright KN, Wu TH & Korba BA (1997) Enhanced oral absorption and antiviral activity of 1-O-octadecyl-sn-glycero-3-phospho-acyclovir and related compounds in hepatitis B virus infection, *in vitro. Biochemical Pharmacology* **53:**1815–1822.

Huggins J, Smee DF., Martinez M & Bray M (1998) Cidofovir (HPMPC) treatment of monkeypox. *Antiviral Research* **37:**A73.

Huggins J, Zhang ZX & Bray M (1999) Antiviral drug therapy of filovirus infections: S-adenosylhomocysteine hydrolase inhibitors inhibit Ebola virus *in vitro* and in a lethal mouse model. *Journal of Infectious Diseases* **179(Suppl 1):**S240–247.

Ignat'ev GM, Strel'tsova MA, Agafonov AP, Kashentseva EA & Prozorovskii NS (1996) [Experimental study of possible treatment of Marburg hemorrhagic fever with desferal, ribavirin, and homologous interferon]. *Voprosy Virusologii* **41:**206–209. Russian.

Ignatyev G, Steinkasserer A, Streltsova M, Atrasheuskaya A, Agafonov A & Lubitz W (2000) Experimental study on the possibility of treatment of some hemorrhagic fevers. *Journal of Biotechnology* **83:**67–76.

Jahrling PB, Geisbert TW, Geisbert JB, Swearengen JR, Bray M, Jaax NK, Huggins JW, Leduc JW & Peters CJ (1999) Evaluation of immune globulin and recombinant interferon-alpha2b for treatment of experimental Ebola virus infections. *Journal of Infectious Diseases* **179(Suppl 1):**S224–234.

Jahrling PB, Hesse RA, Eddy GA, Johnson KM, Callis RT & Stephen EL (1980) Lassa virus infection of rhesus monkeys: pathogenesis and treatment with ribavirin. *Journal of Infectious Diseases* **141:**580–589.

Kern ER (2003) *In vitro* activity of potential anti-poxvirus agents. *Antiviral Research* **57:**35–40.

Kern ER, Hartline C, Harden E, Keith K, Rodriguez N, Beadle JR & Hostetler KY (2002) Enhanced inhibition of orthopoxvirus replication *in vitro* by alkoxyalkyl esters of cidofovir and cyclic cidofovir. *Antimicrobial Agents and Chemotherapy* **46:**991–995.

Kesson AM, Ferguson JK, Rawlinson WD & Cunningham AL (1997) Progressive vaccinia treated with ribavirin and vaccinia immune globulin. *Clinical Infectious Diseases* **25:**911–914.

Kilgore PE, Ksiazek TG, Rollin PE, Mills JN, Villagra MR, Montenegro MJ, Costales MA, Paredes LC & Peters CJ (1997) Treatment of Bolivian hemorrhagic fever with intravenous ribavirin. *Clinical Infectious Diseases* **24:**718–722.

Kirsi JJ, North JA, McKernan PA, Murray BK, Canonico PG, Huggins JW, Srivastava PC & Robins RK (1983) Broad-spectrum antiviral activity of 2-beta-D-ribofuranosylselenazole-4-carboxamide, a new antiviral agent. *Antimicrobial Agents and Chemotherapy* **24:**353–361.

Koplan JP, Monsur KA, Foster SO, Huq F, Rahaman MM, Huq S, Buchanan RA & Ward NA. (1975) Treatment of variola major with adenine arabinoside. *Journal of Infectious Diseases* **131:**34–39.

Licata JM, Simpson-Holley M, Wright NT, Han Z, Paragas J & Harty RN (2003) Overlapping motifs (PTAP and PPEY) within the Ebola virus VP40 protein function independently as late budding domains: involvement of host proteins TSG101 and VPS-4. *Journal of Virology* **77:**1812–1819.

McCormick JB, King IJ, Webb PA, Scribner CL, Craven RB, Johnson KM, Elliott LH & Belmont-Williams R (1986) Lassa fever. Effective therapy with ribavirin. *New England Journal of Medicine* **314:**20–26.

McKee KT Jr, Huggins JW, Trahan CJ & Mahlandt BG (1988) Ribavirin prophylaxis and therapy for experimental argentine hemorrhagic fever. *Antimicrobial Agents and Chemotherapy* **32:**1304–1309.

Meadows KP, Tyring SK, Pavia AT & Rallis TM (1997) Resolution of recalcitrant molluscum contagiosum virus lesions in human immunodeficiency virus-infected patients treated with cidofovir. *Archives of Dermatology* **133:**987–990.

Mitsuya H, Looney DJ, Kuno S, Ueno R, Wong-Staal F & Broder S (1988) Dextran sulfate suppression of viruses in the HIV family: inhibition of virion binding to $CD4^+$ cells. *Science* **240:**646–649.

Moss B (2001) *Fields Virology,* Philadelphia: Lippincott Williams and Wilkins.

Neumann-Haefelin D, Sundmacher R, Sauter B, Karges HE & Manthey KF (1975) Effect of human leukocyte interferon on vaccinia-and herpes virus-infected cell cultures and monkey corneas. *Infection and Immunity* **12:**148–155.

Neyts J, Andrei G, Snoeck R, Jahne G, Winkler I, Helsberg M, Balzarini J & De Clercq E (1994) The N-7-substituted acyclic nucleoside analog 2-amino-7-[(1,3-dihydroxy-2-propoxy)methyl]purine is a potent and selective inhibitor of herpesvirus replication. *Antimicrobial Agents and Chemotherapy* **38:**2710–2716.

Neyts J & De Clercq E (1993) Efficacy of (S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine for the treatment of lethal vaccinia virus infections in severe combined immune deficiency (SCID) mice. *Journal of Medical Virology* **41:**242–246.

Neyts J & De Clercq E (2001) Efficacy of 2-amino-7-(1,3-dihydroxy-2-propoxymethyl)purine for treatment of vaccinia virus (orthopoxvirus) infections in mice. *Antimicrobial Agents and Chemotherapy* **45:**84–87.

Neyts J, Verbeken E & De Clercq E (2002) Effect of 5-iodo-2′-deoxyuridine on vaccinia virus (orthopoxvirus) infections in mice. *Antimicrobial Agents and Chemotherapy* **46:**2842–2847.

Obara T, Shuto S, Saito Y, Snoeck R, Andrei G, Balzarini J, De Clercq E & Matsuda A (1996) New neplanocin analogues. 7. Synthesis and antiviral activity of 2-halo derivatives of neplanocin A. *Journal of Medicinal Chemistry* **39:**3847–3852.

Ott DE, Coren LV, Sowder RC 2nd, Adams J, Nagashima K & Schubert U (2002) Equine infectious anemia virus and the ubiquitin-proteasome system. *Journal of Virology* **76:**3038–3044.

Patil SD, Schneller SW, Hosoya M, Snoeck R, Andrei G, Balzarini J & De Clercq E (1992) Synthesis and antiviral properties of (+/-)-5′-noraristeromycin and related purine carbocyclic nucleosides. A new lead for anti-human cytomegalovirus agent design. *Journal of Medicinal Chemistry* **35:**3372–3377.

Patnaik A, Chau V & Wills JW (2000) Ubiquitin is part of the retrovirus budding machinery. *Proceedings of the National Academy of Sciences of the United States of America* **97:**13069–13074.

Putterman D, Pepinsky RB & Vogt VM (1990) Ubiquitin in avian leukosis virus particles. *Virology* **176:**633–637.

Rao AR, Jacobs ES, Kamalakshi S, Bradbury & Swamy A (1969) Chemoprophylaxis and chemotherapy in variola major. I. An assessment of CG 662 and Marboran in prophylaxis of contacts of variola major. *Indian Journal of Medical Research* **57:**477–483.

Remesar MC, Blejer JL, Weissenbacher MC & Nejamkis MR (1988) Ribavirin effect on experimental Junin virus-induced encephalitis. *Journal of Medical Virology* **26:**79–84.

Rice WG, Turpin JA, Schaeffer CA, Graham L, Clanton D, Buckheit RW Jr, Zaharevitz D, Summers MF, Wallqvist A & Covell DG (1996) Evaluation of selected chemotypes in coupled cellular and molecular target-based screens identifies novel HIV-1 zinc finger inhibitors. *Journal of Medicinal Chemistry* **39:**3606–3616.

Robins MJ, Wnuk SF, Yang X, Yuan CS, Borchardt RT, Balzarini J & De Clercq E (1998) Inactivation of S-adenosyl-L-homocysteine hydrolase and antiviral activity with 5′,5′,6′,6′-tetradehydro-6′-deoxy-6′-halohomoadenosine analogues (4′-haloacetylene analogues derived from adenosine). *Journal of Medicinal Chemistry* **41:**3857–3864.

Safrin S, Cherrington J & Jaffe HS (1997) Clinical uses of cidofovir. *Reviews in Medical Virology* **7:**145–156.

Schubert U, Ott DE, Chertova EN, Welker R, Tessmer U, Princiotta MF, Bennink JR, Krausslich HG & Yewdell JW (2000) Proteasome inhibition interferes with gag polyprotein processing, release, and maturation of HIV-1 and HIV-2. *Proceedings of the National Academy of Sciences of the United States of America* **97:**13057–13062.

Sergeev AN, Ryzhikov AB, Bulychev LE, Evtin NK, P'iankov OV, P'iankova OG, Slezkina EI, Kotliarov LA, Petrishchenko VA & Pliasunov IV (1997) [Study of the treatment-prophylactic effect of immunomodulators in experimental infections, caused by Marburg, Ebola, and Venezuelan equine encephalitis viruses]. *Voprosy Virusologii* **42:**226–229. Russian.

Shuto S, Obara T, Saito Y, Andrei G, Snoeck R, De Clercq E & Matsuda A (1996) New neplanocin analogues. 6. Synthesis and potent antiviral activity of 6'-homoneplanocin A1. *Journal of Medicinal Chemistry* **39:**2392–2399.

Siddiqi SM, Chen X, Raoo J, Schneller SW, Ikeda S, Snoeck R, Andrei G, Balzarini J & De Clercq E (1995) 3-deaza- and 7-deaza-5′-noraristeromycin and their antiviral properties. *Journal of Medicinal Chemistry* **38:**1035–1038.

Siddiqi SM, Chen X, Schneller SW, Ikeda S, Snoeck R, Andrei G, Balzarini J & De Clercq E (1994) An epimer of 5'-noraristeromycin and its antiviral properties. *Journal of Medicinal Chemistry* **37:**1382–1384.

Sidwell RW, Allen LB, Huffman JH, Revankar GR, Robins RK & Tolman RL (1975) Viral keratitis-inhibitory effect of 9-beta-D-arabinofuranosylhypoxanthine 5′-monophosphate. *Antimicrobial Agents and Chemotherapy* **8:**463–467.

Sidwell RW, Allen LB, Khare GP, Huffman JH, Witkowski JT, Simon LN & Robins RK (1973) Effect of 1-beta-D-ribofuranosyl1-1,2,4-triazole-3-carboxamide (virazole, ICN 1229) on herpes and vaccinia keratitis and encephalitis in laboratory animals. *Antimicrobial Agents and Chemotherapy* **3:**242–246.

Sidwell RW, Dixon GJ, Sellers SM & Schabel FM Jr (1968) *In vivo* antiviral properties of biologically active compounds. II. Studies with influenza and vaccinia viruses. *Applied Microbiology* **16:**370–392.

Sidwell RW, Huffman JH, Khare GP, Allen LB, Witkowski JT & Robins RK (1972) Broad-spectrum antiviral activity of Virazole: 1-beta-D-ribofuranosyl-1,2,4-triazole-3-carboxamide. *Science* **177:**705–706.

Smee DF, Bailey KW, Wong M & Sidwell RW (2000) Intranasal treatment of cowpox virus respiratory infections in mice with cidofovir. *Antiviral Research* **47:**171–177.

Smee DF & Sidwell RW (2003) A review of compounds exhibiting anti-orthopoxvirus activity in animal models. *Antiviral Research* **57:**41–52.

Sodeik B, Griffiths G, Ericsson M, Moss B & Doms RW (1994) Assembly of vaccinia virus: effects of rifampin on the intracellular distribution of viral protein p65. *Journal of Virology* **68:**1103–1114.

Streeter DG, Witkowski JT, Khare GP, Sidwell RW, Bauer RJ, Robins RK & Simon LN (1973) Mechanism of action of 1- -D-ribofuranosyl-1,2,4-triazole-3-carboxamide (Virazole), a new broad-spectrum antiviral agent. *Proceedings of the National Academy of Sciences of the United States of America* **70:**1174–1178.

Thompson RL, Minton SA Jr, Officer JE & Hitchings GH (1953) Effect of heterocyclic and other thiosemicarbazones on vaccinia infection in the mouse. *Journal of Immunology* **70:**229–234.

Towner JS, Rollin PE, Bausch DG, Sanchez A, Crary SM, Vincent M, Lee WF, Spiropoulou CF, Ksiazek TG, Lukwiya M, Kaducu F, Downing R & Nichol ST (2004) Rapid diagnosis of Ebola hemorrhagic fever by reverse transcription-PCR in an outbreak setting and assessment of patient viral load as a predictor of outcome. *Journal of Virology* **78:**4330–4341.

Tseng CK, Marquez VE, Fuller RW, Goldstein BM, Haines DR, McPherson H, Parsons JL, Shannon WM, Arnett G, Hollingshead M & *et al* (1989) Synthesis of 3-deazaneplanocin A, a powerful inhibitor of S-adenosylhomocysteine hydrolase with potent and selective *in vitro* and *in vivo* antiviral activities. *Journal of Medicinal Chemistry* **32:**1442–1446.

Tummino PJ, Harvey PJ, McQuade T, Domagala J, Gogliotti R, Sanchez J, Song Y & Hupe D (1997) The human immunodeficiency virus type 1 (HIV-1) nucleocapsid protein zinc ejection activity of disulfide benzamides and benzisothiazolones: correlation with anti-HIV and virucidal activities. *Antimicrobial Agents and Chemotherapy* **41:**394–400.

Uckun FM, Chen CL, Lisowski E, Mitcheltree GC, Venkatachalam TK, Erbeck D, Chen H & Waurzyniak B (2003) Toxicity and pharmacokinetics of stampidine in mice and rats. *Arzneimittel-Forschung* **53:**357–367.

Uckun FM, Petkevich AS, Vassilev AO, Tibbles HE & Titov L (2004) Stampidine prevents mortality in an experimental mouse model of viral hemorrhagic fever caused by lassa virus. *BMC Infectious Diseases* **4:**1.

Van Aerschot AA, Mamos P, Weyns NJ, Ikeda S, De Clercq E & Herdewijn PA (1993) Antiviral activity of C-alkylated purine nucleosides obtained by cross-coupling with tetraalkyltin reagents. *Journal of Medicinal Chemistry* **36:**2938–2942.

Vlasuk GP & Rote WE (2002) Inhibition of factor VIIa/tissue factor with nematode anticoagulant protein c2: from unique mechanism to a promising new clinical anticoagulant. *Trends in Cardiovascular Medicine* **12:**325–331.

Vogt VM (2000) Ubiquitin in retrovirus assembly: actor or bystander? *Proceedings of the National Academy of Sciences of the United States of America* **97:**12945–12947.

Votruba I, Bernaerts R, Sakuma T, De Clercq E, Merta A, Rosenberg I & Holy A (1987) Intracellular phosphorylation of broad-spectrum anti-DNA virus agent (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine and inhibition of viral DNA synthesis. *Molecular Pharmacology* **32:**524–529.

Wachsman M, Petty BG, Cundy KC, Jaffe HS, Fisher PE, Pastelak A & Lietman PS (1996) Pharmacokinetics, safety and bioavailability of HPMPC (cidofovir) in human immunodeficiency virus-infected subjects. *Antiviral Research* **29:**153–161.

Walton E, Jenkins SR, Nutt RF & Holly FW (1969) Branched-chain sugar nucleosides. V. Synthesis and antiviral properties of several branched-chain sugar nucleosides. *Journal of Medicinal Chemistry* **12:**306–309.

Weissenbacher MC, Avila MM, Calello MA, Merani MS, McCormick JB & Rodriguez M (1986a) Effect of ribavirin and immune serum on Junin virus-infected primates. *Medical Microbiology and Immunology* **175:**183–186.

Weissenbacher MC, Calello MA, Merani MS, McCormick JB & Rodriguez M (1986b) Therapeutic effect of the antiviral agent ribavirin in Junin virus infection of primates. *Journal of Medical Virology* **20:**261–267.

Witvrouw M, Balzarini J, Pannecouque C, Jhaumeer-Laulloo S, Este JA, Schols D, Cherepanov P, Schmit JC, Debyser Z, Vandamme AM, Desmyter J, Ramadas SR & De Clercq E (1997) SRR-SB3, a disulfide-containing macrolide that inhibits a late stage of the replicative cycle of human immunodeficiency virus. *Antimicrobial Agents and Chemotherapy* **41:**262–268.

Witvrouw M & De Clercq E (1997) Sulfated polysaccharides extracted from sea algae as potential antiviral drugs. *General Pharmacology* **29:**497–511.

Woodson B & Joklik WK (1965) The inhibition of vaccinia virus multiplication by isatin-beta-thiosemicarbazone. *Proceedings of the National Academy of Sciences of the United States of America* **54:**946–953.

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